The Characterization of Lipopolysaccharide of *Pseudomonas stutzeri P-16* and *Pseudomonas saccharophila P-15*

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INTRODUCTION

Coal gasification and other industrial processes have produced coal tar, a potential carcinogen due to its chemical composition. This byproduct, which is also a component in asphalt and roof sealant, has been leaking into the environment contaminating ground water. Coal tar is a dense non-aqueous phase liquid (DNAPL), which causes it to pool below the water table and causes constant contamination of groundwater (Hatheway, 1997). Since coal tar is a very viscous mixture, it is difficult to clean up and so biodegradation may be one promising technology to reduce the concentration of this environmental pollutant.

Pseudomonas stutzeri P-16 and *Pseudomonas saccharophila P-15* are two bacteria that use polycyclic aromatic hydrocarbons (PAH) present in coal tar as an energy source and carbon source. PAH biodegradation might be enhanced via organisms directly association with the DNAPL. Bacterial/ DNAPL interactions depend on bacteria and DNAPL surface properties. The overall hypothesis of this research is that bacterial membrane properties can be influenced by growth conditions to maximize bacterial adhesion at DNAPL interfaces. Prior studies have shown that P-16 and P-15 adhesion to hexadecane depends on growth conditions. The objective of this research is to determine the effect of growth condition on the composition of one group of membrane bound compounds. Lipopolysaccharide (LPS) are composed of a sugar and lipid extending off of the bacteria's gram-negative membrane, which enables the bacteria to interact with DNAPL. The altered bacteria can change the rate of degradation according to its success in adhering to the DNAPL. The relationship of LPS composition in response to growth condition may allow LPS to attach to coal tar more successfully, which will in turn increase the rate of degradation.

METHODS AND MATERIALS

1. Growth of bacteria

Solutions of peptone, glucose, naphthalene, and coal tar were inoculated with both *Pseudomonas*

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species. The solutions were incubated at 30°C until they met an absorbance reading of 0.6-0.8 at 420nm. The bacteria were harvested at 7,000 rpm for 15 min and the pellet was lyophilized.

2. LPS Recovery

LPS was secured using three different methods: phenol extraction, long Ethylenediaminetetra-acetic acid (EDTA) extraction, and short EDTA extraction. The LPS characteristics from each method were obtained to find the best way to extract the LPS for each media type. The short EDTA method involved combining lyophilized *Pseudomonas* with a Tris-buffer and centrifuging. The supernatant was dialyzed against deionized water over night and lyophilized. The product was purified with sepharose gel-filtration. Phenol-water extraction implements a heating and cooling method with phenol to extract the LPS. The product is purified using dialysis and centrifugation. The pellet is then lyophilized to yield pure LPS. Long EDTA utilizes Dnase, RNase, sonication, incubation, and EDTA to free the LPS from the membrane. The crude LPS is purified using sonication and ultracentrifugation.

3. Characterizing the LPS

Multiple methods may be used to depict the extracted LPS according to the growth media and extraction method used. The concentration, the protein structure, and the interfacial tension may change. Using a dye method, which stains the whole LPS chain, the concentration of LPS extracted from each method was found by creating a standard curve with known LPS concentration versus absorbance at 472 nm. Through sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), the molecular weight of the LPS is defined through the break down of the proteins that compose the LPS into individual polypeptide chains (Laemmli,1970). A 10-12% stacking gel is created and LPS combined with sample buffer are placed in the gel. 190-200 volts are applied, then the gel is stained making the molecular weights of each chain visible. The cell surface properties may affect the interfacial tension between the coal tar and the water because the presence of LPS. Using a tensiometer, the interfacial tension between hexadecane and water has been quantified.

RESULTS AND DISCUSSION

Using the dye method standard curve, shown in Figure 1, concentrations of 0.0153µg/0.5 ml and 0.186µg/0.5 ml were obtained using the short EDTA method. These values demonstrate that some LPS had been removed successfully, which allowed for the characterization of LPS with interfacial tension. The interfacial tension between hexadecane and buffer with LPS is significantly lower than the interfacial tension between hexadecane and buffer without LPS. The average interfacial tension (IFT) value with LPS was 19.81±0.1212 dynes/cm and without LPS was 43.37±1.1425 dynes/cm. This result displays the effect of the LPS; the lower IFT will increase surface area and therefore enhance PAH dissolution, which will result in enhanced biodegradation. This will increase coal tar mobility making it more susceptible to come into contact with more coal tar degrading bacteria and will allow the rate of biodegradation to increase.

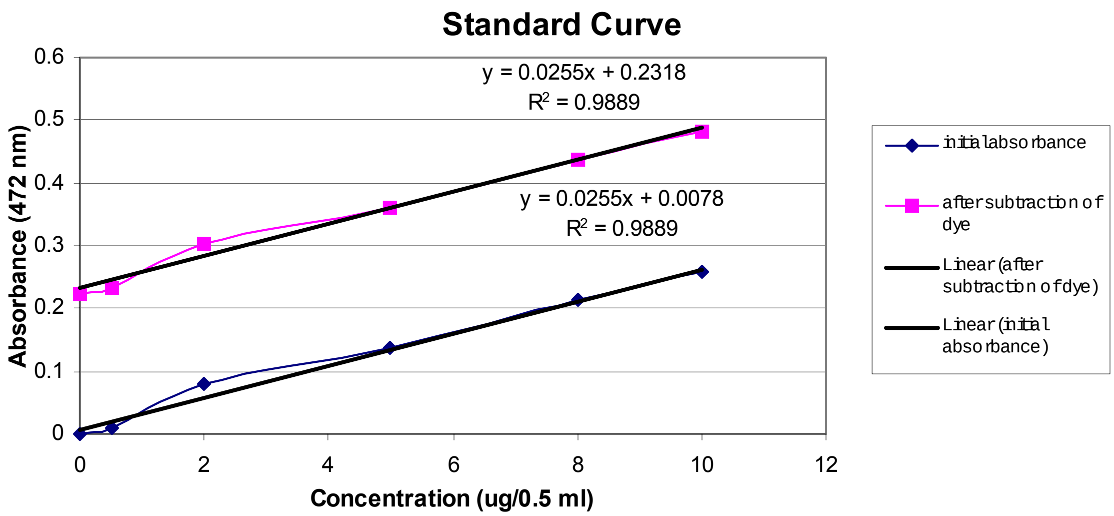

Figure 1. The standard curve for the dye method at LPS concentrations of 0, 0.5, 2.0,5.0,8.0,10.0µg/0.5 ml DI water taken at 472 nm.

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